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ID-1-P-3104 SCANNING ELECTRON MICROSCOPY OF LIVE-CELLS ENABLED BY IN-SITU FLUORESCENCE MICROSCOPY

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One of the major strengths of light microscopy techniques, and especially fluorescence microscopy (FM), is the ability to observe rare dynamic events, in vitro as well as in vivo. A main challenge, however, is to image the positions of labelled proteins with respect to cellular ultrastructure during such an event with a resolution beyond that of the light microscope. Electron microscopy (EM) of samples in a near-native (buffer) environment has recently been shown possible, but the destructive nature of EM precludes observing cellular dynamics. We present a novel approach towards dynamic bio-imaging wherein live-cell FM is carried out in-situ in a scanning electron microscope (SEM) (Figure 1). This allows us to capture structural SEM snapshots on-demand based on the FM observations.

We study the uptake and retrograde transport of EGF-conjugated quantum dots (QDots) in filopodia of fibroblasts. The cells are cultured on a thin, electron-transparent substrate, which is then placed in a light-transparent sample holder containing the EGF-QDot solution. This sample holder is mounted in an SEM with integrated high-numerical aperture fluorescence microscope. The uptake process is monitored with FM (Figure 2) and based on these observations, we determine both the desired moment for the SEM image and the location of filopodia within the SEM field of view. These SEM images show the positions of individual QDots on the cytoskeleton transport tracks within filopodia from the position of uptake up to the docking region at the microtubules frontier where Qdots accumulate before further transport towards the nucleus.

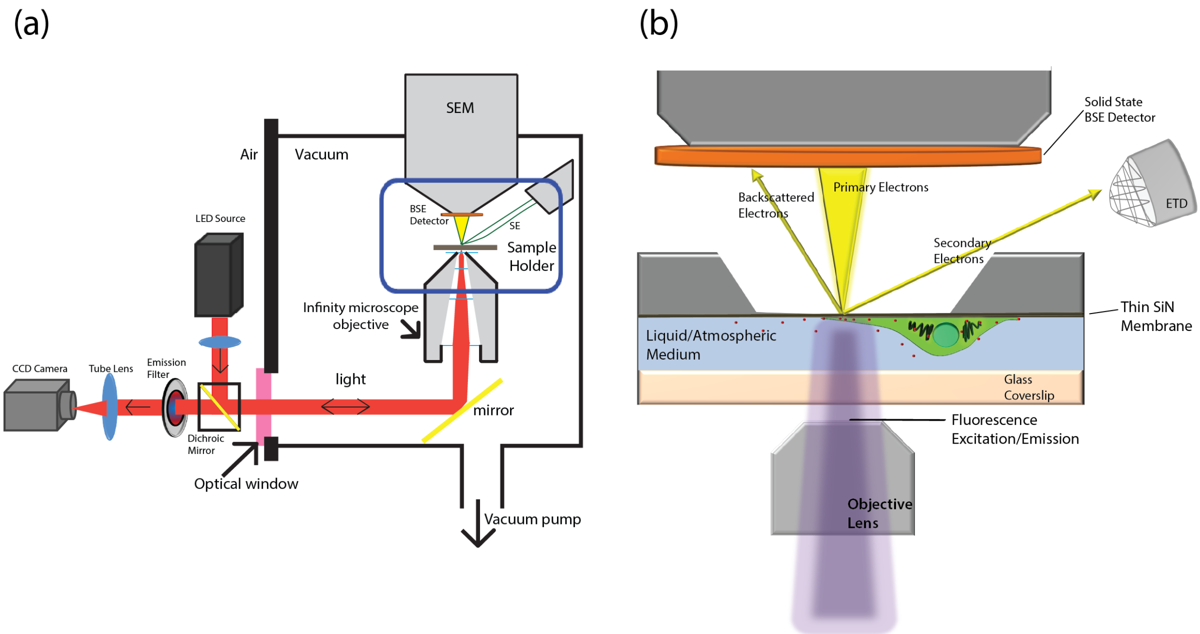

Fig. 1: (a) Schematic of the Scanning Electron Microscope with in-situ epi-fluorescence microscope. The fields of view are aligned such that an area on the sample can be observed with both microscopes. (b) Illustration showing a live cell contained in liquid in a capsule with electron and light transparent windows mounted in the in-situ microscope

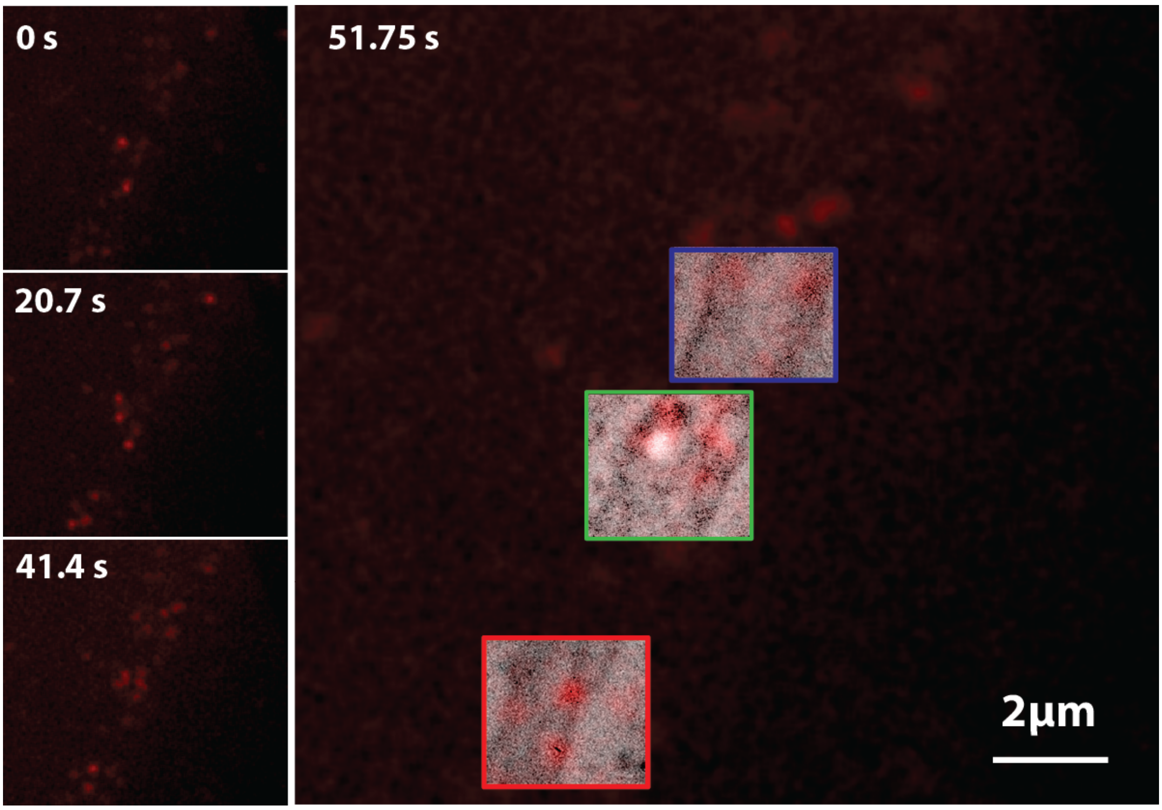

Fig. 2: Snapshots from a fluorescence movie showing transport of quantum dots conjugated to epi-dermal growth factor in a cellular extension. After 51.75 seconds observation time, SEM snapshots were taken, which are shown overlaid to the fluorescence image in the main panel.